

# A STUDY OF THE LIFE HISTORY OF TRILLIUM CERNUUM L.

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(WITH PLATE XXVII)

The following study of the origin and development of the female gametophyte of *Trillium cernuum* has been made with a view to a more detailed cytological and embryological study of *T. cernuum* and *T. grandiflorum* and their possible hybrids. It was undertaken at the suggestion of Dr. FERGUSON, to whom I am greatly indebted for helpful discussion and criticism.

ATKINSON<sup>1</sup> has given a careful description of microsporogenesis in *T. grandiflorum*. ERNST<sup>2</sup> has published a very brief and as yet uncompleted account of chromosome reduction, development of the embryo sac, and fertilization in *T. grandiflorum*. GRÉGOIRE and WYGAERTS<sup>3</sup> have used *T. cernuum* and *T. grandiflorum* as a basis for detailed studies on the reconstruction of the nucleus and the formation of chromosomes. As yet, however, no account of the life history of *T. cernuum* has been given.

*Trillium cernuum* is a native of Massachusetts and grows in sufficient abundance in the vicinity of Wellesley College to furnish plenty of material for investigation. The work of collecting was done in 1911 and 1912. From the first week in April until the end of May, material was gathered once or twice each day. The fixing was generally done in the field. The most satisfactory fixing agent was a weak solution of Flemming's chromo-acetic-osmic fluid. Of the several stains tried, Haidenhain's iron-alum-hematoxylin and Flemming's triple stain gave the best results.

<sup>1</sup> ATKINSON, G. F., Studies on reduction in plants. BOT. GAZ. 28:1-24. pls. 3-6. 1899.

<sup>2</sup> ERNST, A., Chromosomenreduction, Entwicklung des Embryosackes, und Befruchtung bei *Paris quadrifolia* und *Trillium grandiflorum*. Flora 91:1-36. pls. 1-6. 1902.

<sup>3</sup> GRÉGOIRE, VICTOR, and WYGAERTS, A., La reconstitution du noyau et la formation des chromosomes dans les cinèse somatiques. La Cellule 21:7-76. pls. 2. 1904.

By the end of September the flower buds are well formed and the parts of the ovules are clearly differentiated (fig. 1). An arche-sporial cell immediately below the epidermis has divided and given rise to a primary parietal and a megaspore mother cell (fig. 2). In some ovules the primary parietal cell remains undivided (fig. 2), while in others it divides longitudinally (fig. 1). ERNST (*loc. cit.*) finds no formation of primary parietal tissue in *T. grandiflorum*.

During the winter, the megaspore mother cell of *T. cernuum* is in a resting state and is easily distinguishable from the surrounding cells because of its larger size and its larger and more deeply staining nucleus (figs. 1, 2). If meiosis is accepted as the criterion for spore formation, the fact that this cell later undergoes the reduction divisions proves it to be a megaspore mother cell. The physiologically analogous cell of *T. grandiflorum* is called by ERNST (*loc. cit.*) the embryo sac mother cell. He says: "Die Embryo-sackmutterzelle differenziert sich in der subepidermalen Zellschicht unmittelbar unten dem Scheitel des Nucellus." Since, as in *T. cernuum*, this cell does not give rise directly to the embryo sac, it cannot be the true embryo sac mother cell, and this terminology should be discarded.

With the resumption of growth in late March or early April, active cell division may be observed in all parts of the flower. The resting reticulum of the megaspore mother cell gradually loses its netlike structure and resolves itself into a dense synaptic mass (fig. 3). Later stages show the chromatin threads to have thickened, shortened, and segmented transversely into distinct chromosomes (fig. 4). After a second contraction of the nuclear material (fig. 5) the separate chromosomes soon become arranged at the nuclear plate, while delicate spindle fibers can be distinguished in the cytoplasm (fig. 6).

Anaphase and telophase are quickly passed through, as ERNST (*loc. cit.*) has reported for *T. grandiflorum*, and each of the resulting daughter nuclei becomes invested with a delicate nuclear membrane. The chromosomes of each nucleus seem to fuse end to end to form a long, thick, loosely wound band, but there is no evidence from the material studied that they completely lose their identity at this time and form a reticulum. During this short resting

period, the cell plate gradually becomes heavier and extends across the entire cell as a dividing wall (fig. 7). Every step of this division clearly indicates that it is the heterotypic phase of meiosis.

Although the daughter cells are at first apparently similar in all respects, the chalazal one gradually shows signs of greater vitality, and by more rapid growth encroaches upon the micropylar cell (figs. 8-13). In no case have I found the outer cell giving evidence of being the more vigorous. It may disintegrate immediately (fig. 8); it may pass through one or more phases of the homotypic division (figs. 9-11); or it may even complete meiosis before disorganizing (figs. 12-14). A study of many ovules shows that no cell wall is ever laid down, and that even though the spores may be formed they never become functional, but disintegrate early (fig. 15).

At the completion of the homotypic division in the chalazal cell, no cell wall is formed, and the resulting 2 nuclei undergo a period of rest lasting 2 or 3 days. The cell increases rapidly in size and becomes very vacuolate (fig. 16). The formation of a large, central vacuole soon forces the 2 nuclei to opposite ends of the cell. It is very evident that this structure is a young embryo sac, and thus the female gametophyte is derived from 2 megaspores. ERNST states that 2 megaspores enter into the formation of the embryo sac of *T. grandiflorum*. COULTER and CHAMBERLAIN<sup>4</sup> describe an axial row of 4 megaspores for *Trillium* and report that in *T. recurvatum* the embryo sac is derived from the chalazal megaspore. A study of CHAMBERLAIN'S figures for *T. recurvatum* reveals striking resemblances to those for *T. grandiflorum* and *T. cernuum*. As in the last two named species, there is an axial row of 2 binucleate cells which have resulted from the "second division of the nucleus of the mother cell." No sketch of the 4-celled axial row mentioned in his text is given. The next stage illustrated is that of the young 2-celled embryo sac capped at the micropylar end by a dense, contracted mass, "the remains of the other 3 megaspores." Fig. 15 of this paper shows a very similar embryo sac, but an examination of the preceding stages illustrated makes it clear that the

<sup>4</sup> COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of the angiosperms. Chicago. 1901.

disintegrating masses of tissue represent the remains of only 2 megaspores. Even though it may be true that the number of cells in the axial row varies in closely related species of the same genus, this does not seem to be the case for the 3 species of *Trillium* under discussion. The close resemblance of the series of figures given for each of the 3 species and the fact that CHAMBERLAIN does not figure a 4-celled axial row make it highly probable that *T. recurvatum*, like *T. grandiflorum* and *T. cernuum*, has an axial row of 2 binucleate cells, and that the first 2 cells of its embryo sac represent 2 megaspores. During a summer session which I spent at the University of Chicago in 1912, Dr. CHAMBERLAIN very kindly examined my slides covering these stages and agreed with this interpretation.

After a period of growth of the young embryo sac, the 2 nuclei divide rapidly to form a 4-nucleate, and then an 8-nucleate, embryo sac (figs. 17-19). These phenomena and those attending the maturation of the embryo sac agree so closely with the account already given by ERNST for *T. grandiflorum* as to make further comment on them unnecessary.

From the foregoing account it may be seen that *T. grandiflorum* and *T. cernuum*, the 2 species to be used in the work of hybridizing, agree in all important respects regarding the origin and the development of the embryo sac. The chief points of difference in the two accounts are as follows: (1) one row of primary parietal tissue is formed in the ovules of *T. cernuum*, while in *T. grandiflorum* primary parietal tissue is entirely wanting; (2) in *T. cernuum* the 2 nuclei resulting from the heterotypic division do not form resting reticula.

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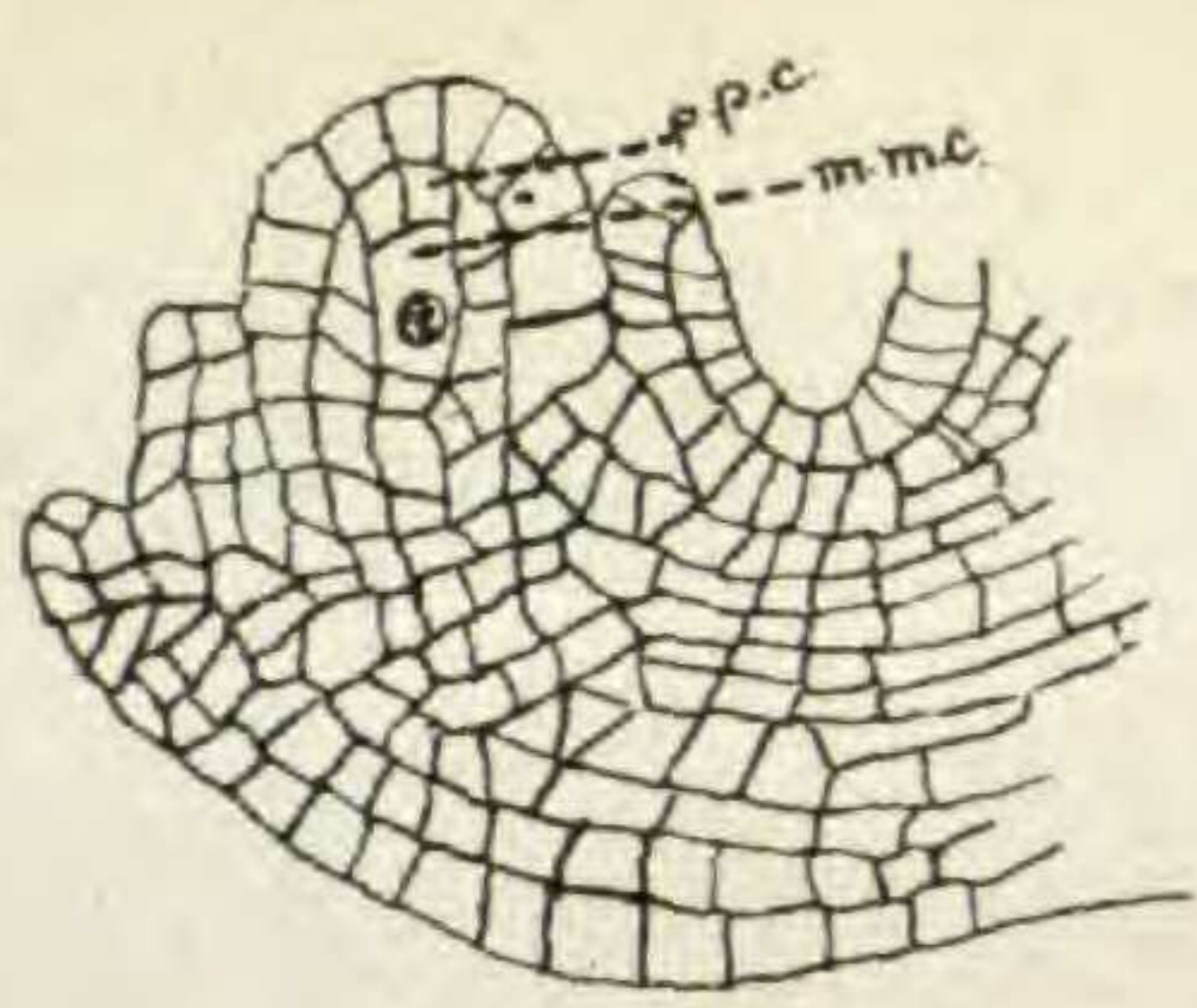
#### EXPLANATION OF PLATE XXVII

An Abbé camera lucida was used for all of the drawings. The figures are oriented with the micropylar end toward the upper edge of the page. The abbreviations used in labeling the drawings are as follows: *m.m.c.*, megaspore mother cell; *p.p.c.*, primary parietal cell.

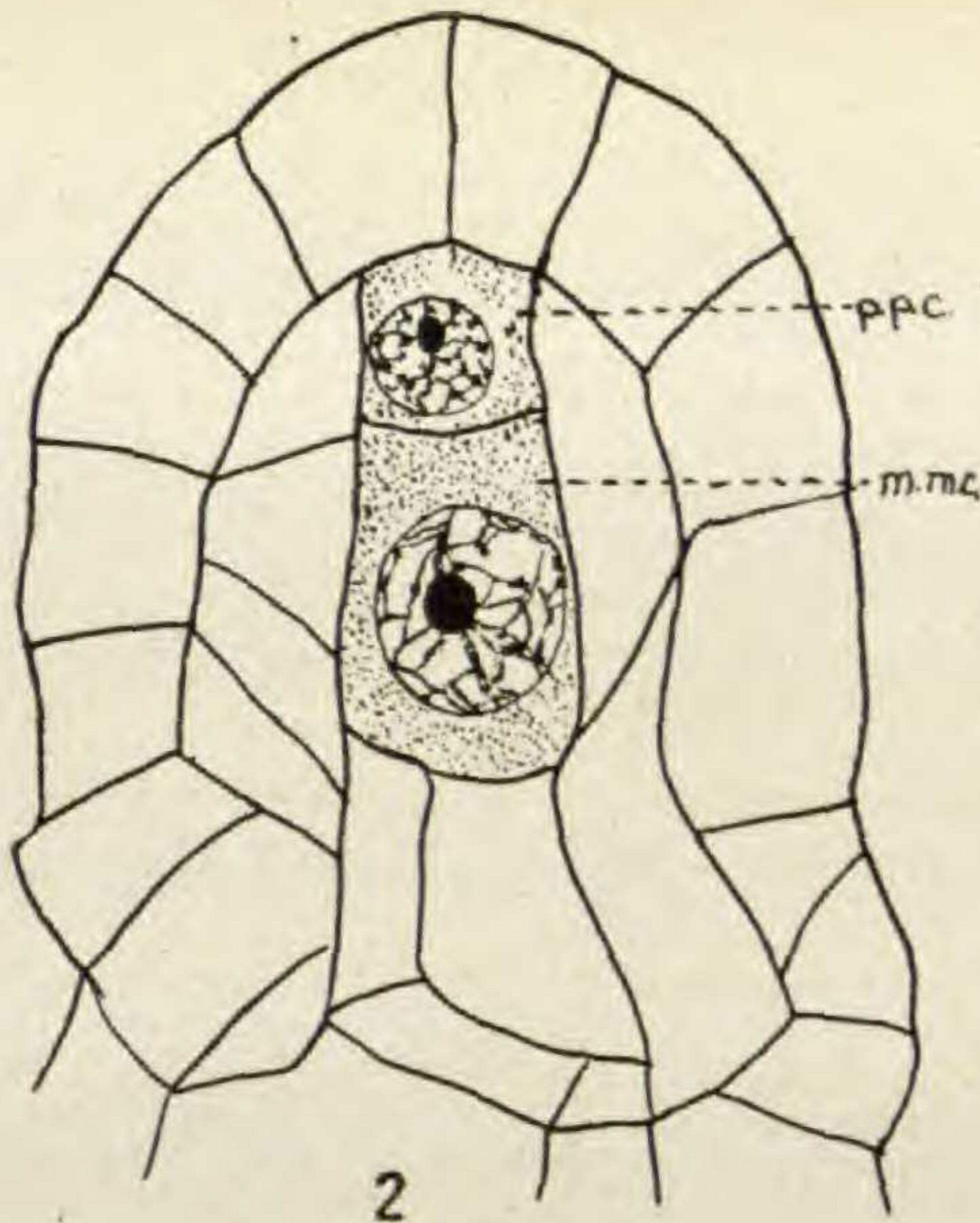
FIG. 1.—Longitudinal section of a young ovule;  $\times 73$ .

FIG. 2.—Same as fig. 1;  $\times 430$ .

FIG. 3.—Megaspore mother cell in synapsis;  $\times 430$ .



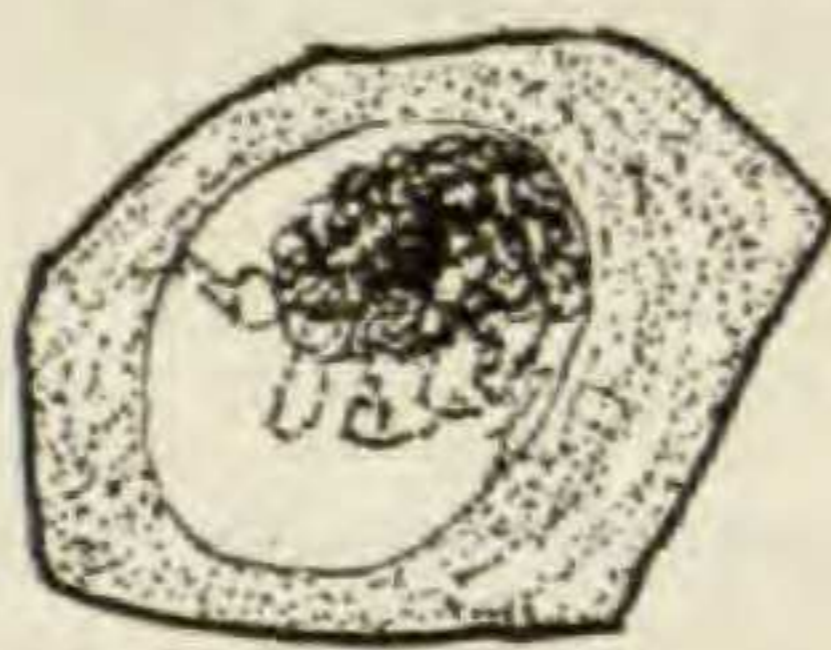
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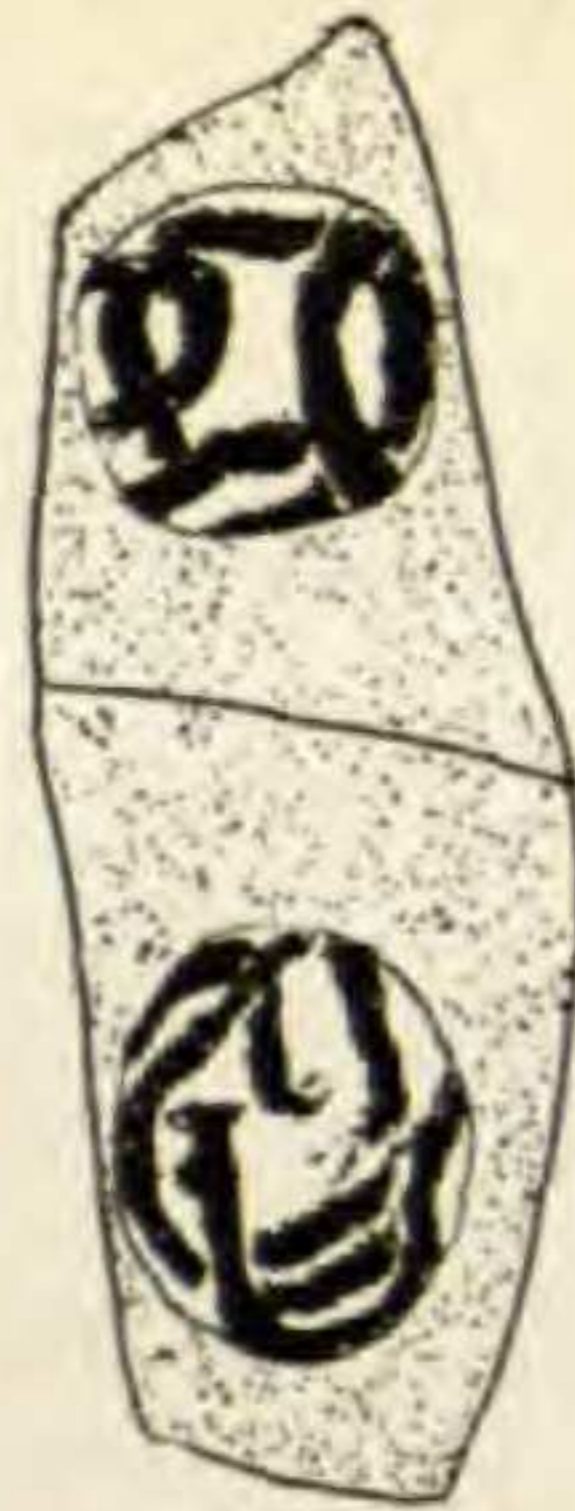
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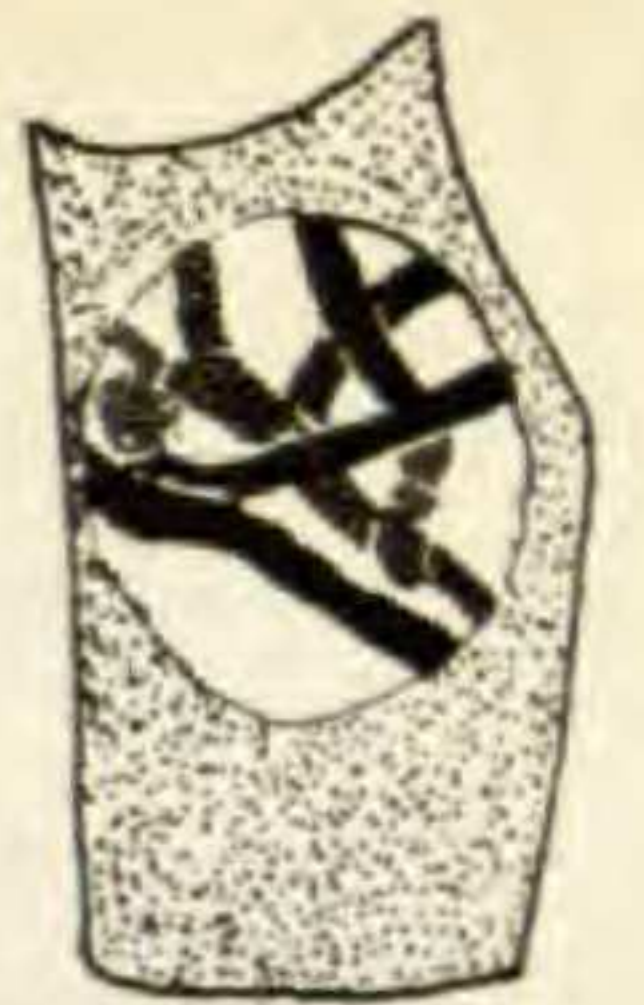
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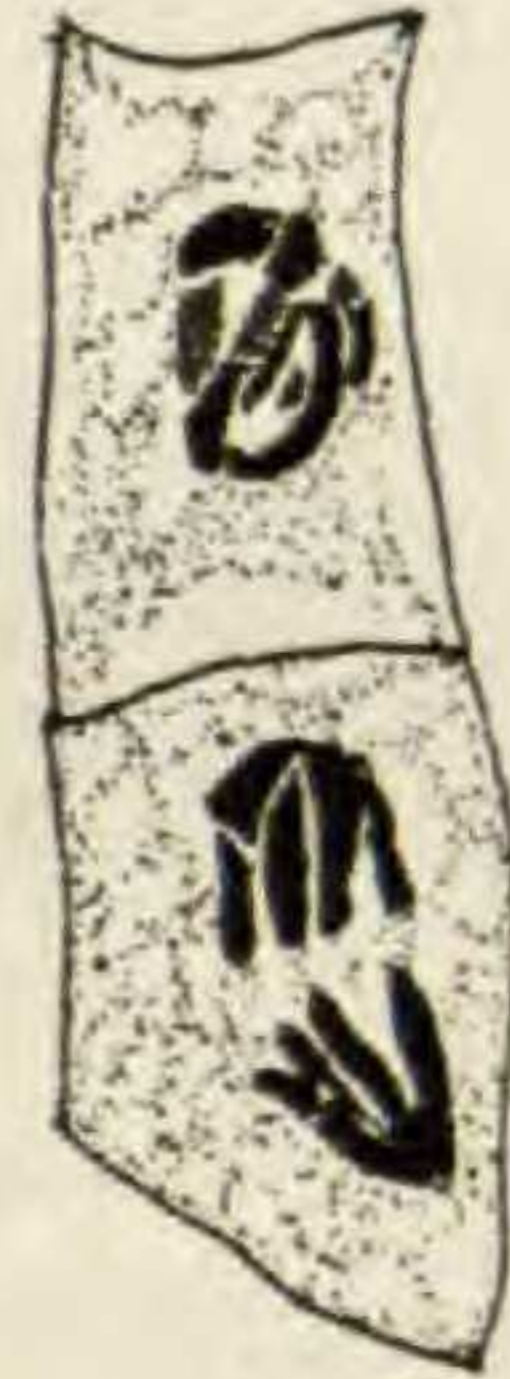
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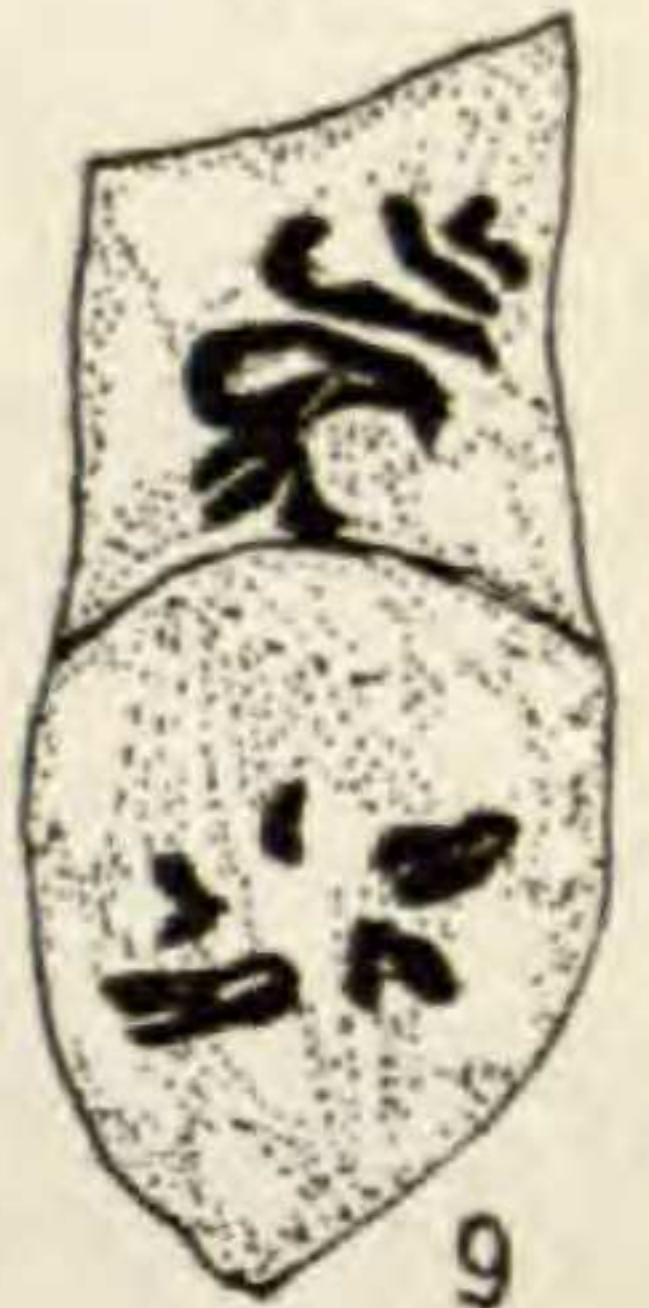
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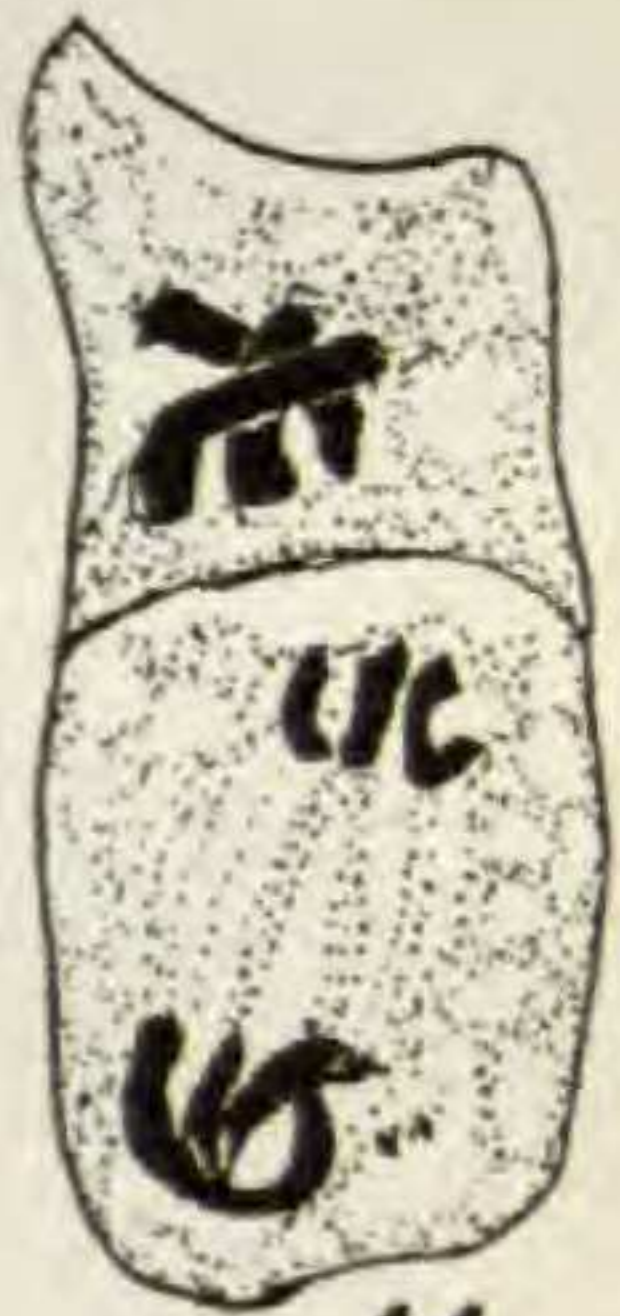
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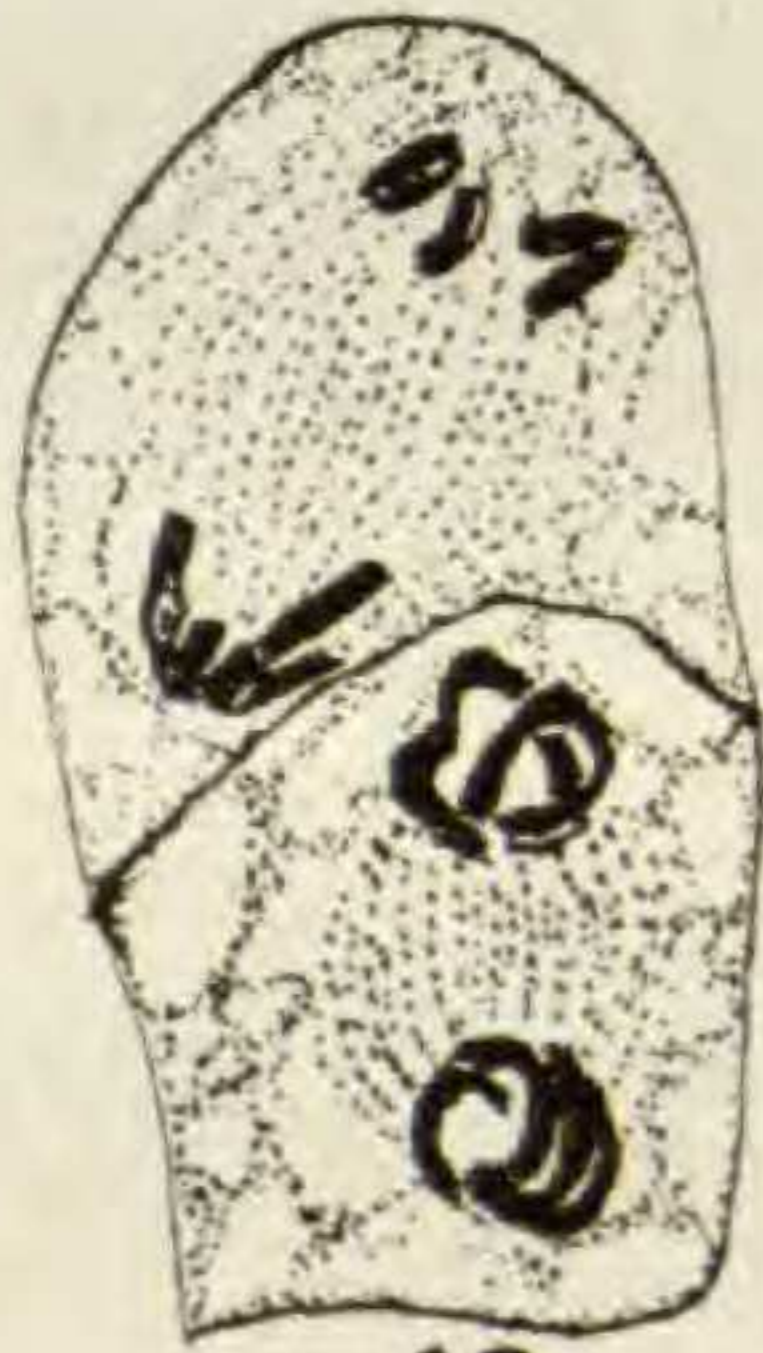
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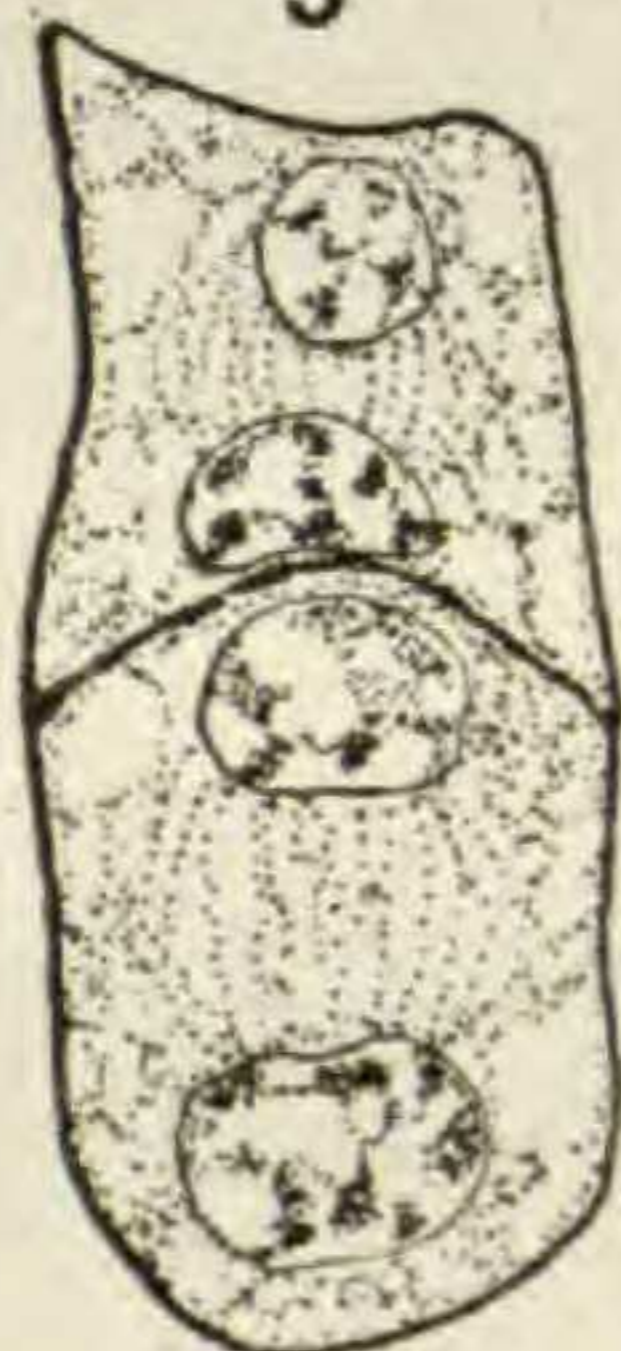
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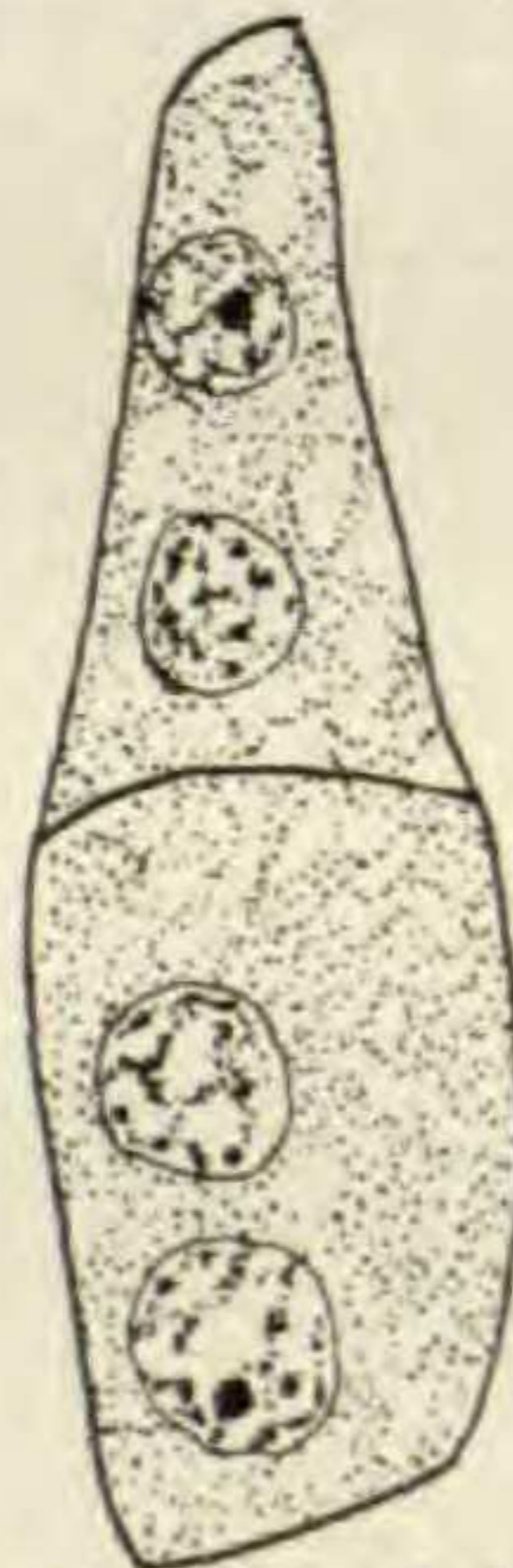
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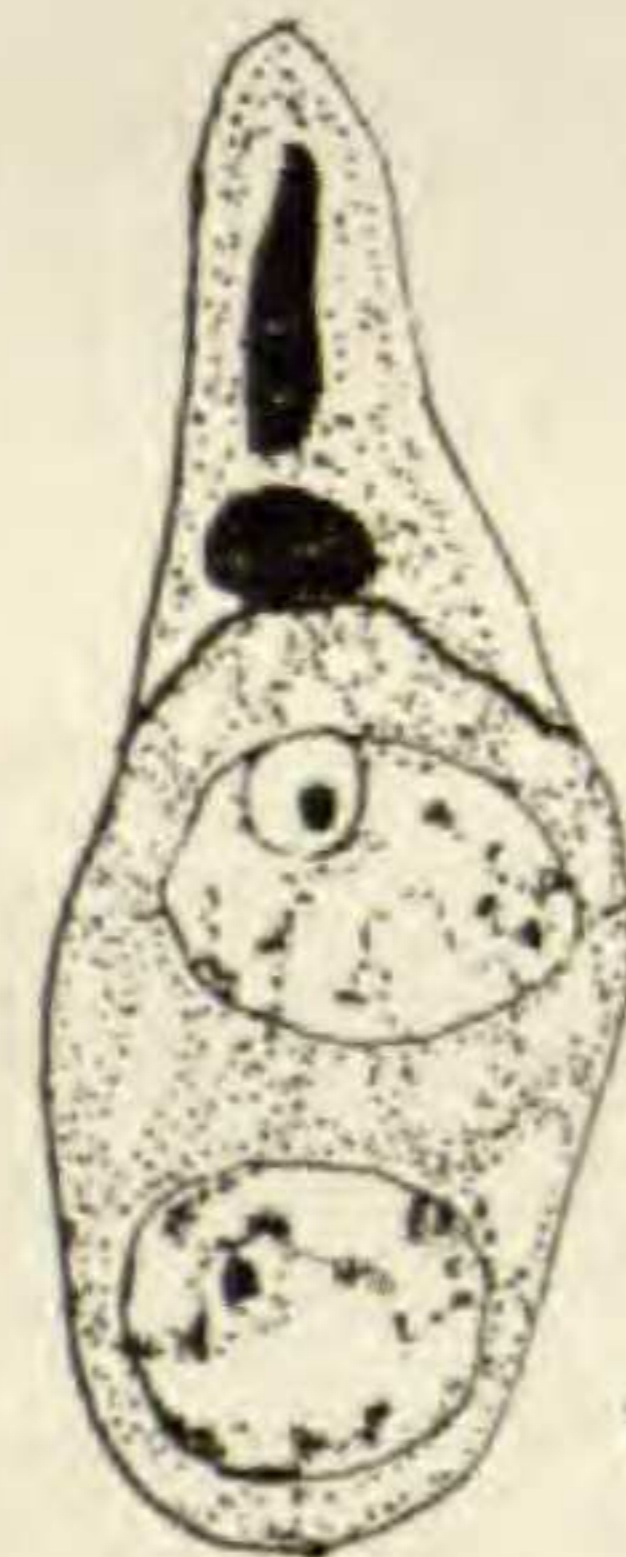
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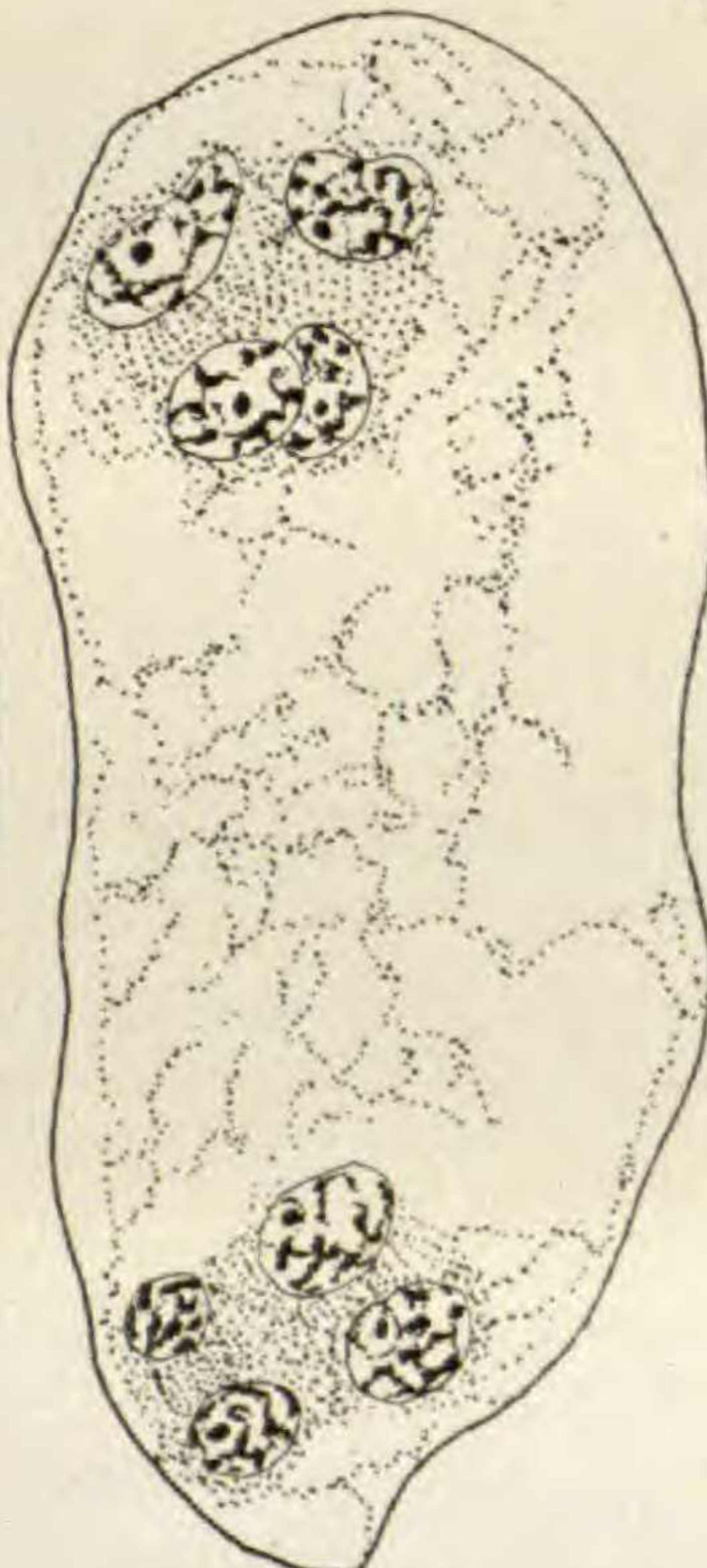
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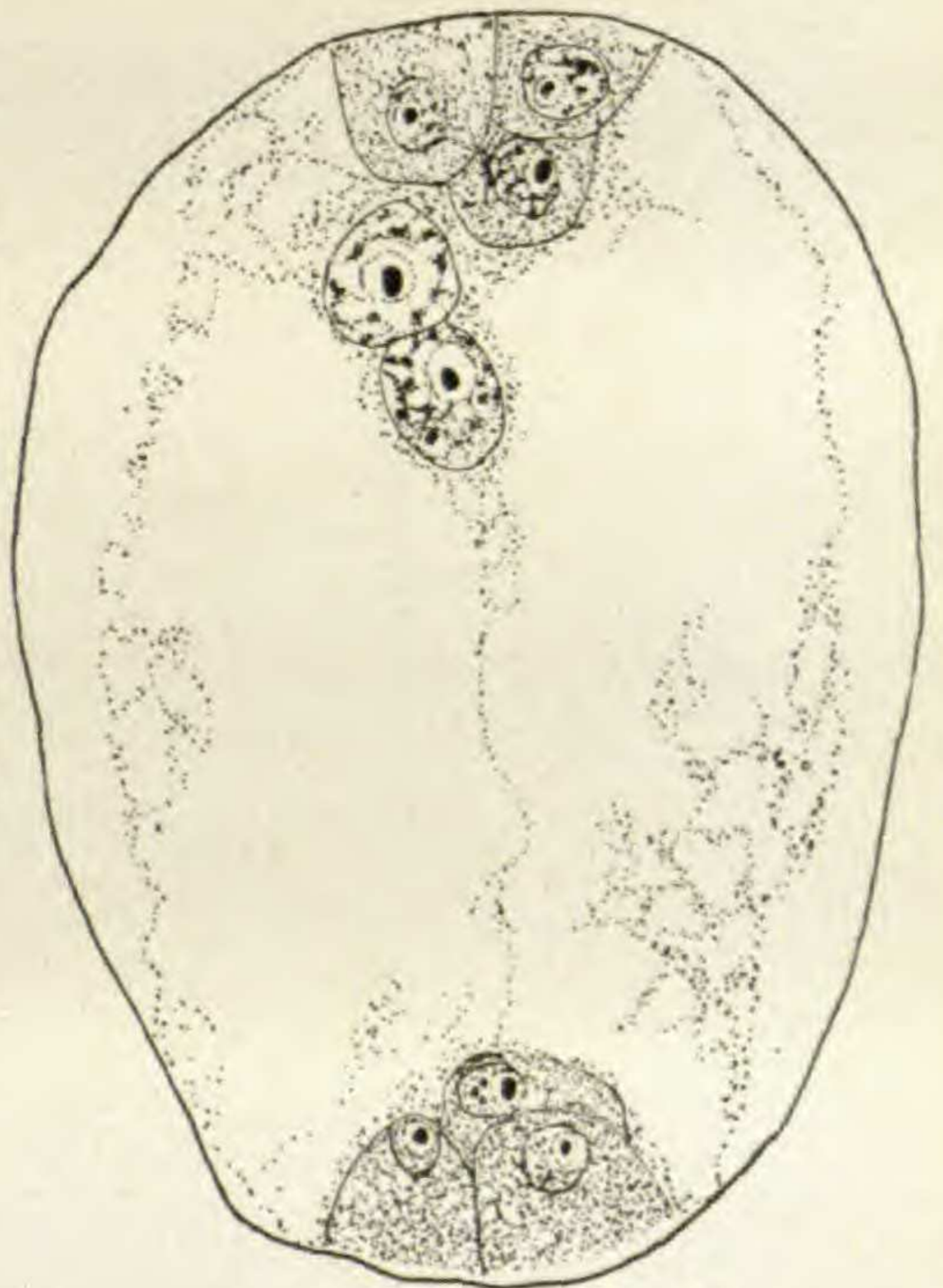
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